Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo **Menthol** No Date

H20

Investigation of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo

MENTHOL

Protocol:

Menthol was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in oil as the solvent by the two routes at two stages of embryonic development: via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (1, 2).

Groups of 10 or more eggs were treated under these four conditions at several dose levels until a total of ninety to one hundred eggs per level was reached for all levels allowing some hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. All hatched chicks and non-viable embryos were examined carefully for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of the test.

Columns 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram, respectively (the milligrams per kilogram figure is based on an average egg weight of fifty grams). Column 3 is the total

number of eggs treated. Column 4 is the percent mortality i.e. total non-viable divided by total treated eggs. Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia, ataxia or other nerve disorders. Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent treated eggs and the untreated controls.

The mortality data in Column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (3). The results obtained are indicated at the bottom of each table.

The data of Columns 4, 5, and 6 have been analyzed using the Chi Square Test for significant differences from the control background. Each dose level is compared to the control value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

At hatchings, 3 chicks were removed at random from each level including control for skeletal clearing, weighing and fixing of bursa, spleen, liver and kidney. Tissues were processed, blocked in paraffin, sectioned, affixed to slides, and stained. Later these sections were examined for internal damage to the tissues.

Discussion:

Menthol was tested at dose levels between 3.00 and 100 mg/kg for all four conditions of the test. The estimated LD-50 values for all the four treatments were as follows: Air cell treatment 00 hours 57.28 mg/kg (2.864 mg/egg); Air cell treatment 96 hours 52.16 mg/kg (2.608 mg/egg); Yolk treatment 00 hours 11.42 mg/kg (0.571 mg/egg); and Yolk treatment for 96 hours 72.41 mg/kg (3.6205 mg/egg).

By the two routes (air cell and yolk) and at two stages of embryonic development, Menthol was toxic when administered at 50 mg/kg and above. Even at the level of 10 mg/kg, menthol was toxic when administered at 00 hours via yolk. However, there were no significant abnormalities observed at any dose level in all the four treatments.

References:

1. McLaughlin, J., Jr., Marliac, J.-P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O. G., (1965) Toxicol. Appl. Pharmacol. 5, 760-770.

2. Verrett, M. J., Marliac, J.-P., and McLaughlin, J., Jr., (1964) JAOAC 47, 1003-1006.

3. Finney, D. J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendic I.

MENTHOL
AIR CELL 0 HOURS

DOSE mg/egg	DOSE mg/kg	Number of Eggs	Percent Mortality *	Percent Abnormal Total	Percent Abnormal Structural
5.00	100.00	98	66.32*	0.0	0.0
3.75	75.00	100	65.00*	0.0	0.0
2.50	50.00	100	49.00*	0.0	0.0
0.50	10.00	98	37.75	1.02	0.0
0.15	3.00	100	19.00	0.0	0.0
Sesame Oil		50	28.00	0.0	0.0

*Significantly different from solvent $p \leq 0.05$

MENTHOL
AIR CELL 96 HOURS

Dose		Number of Eggs	Percent Mortality*	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
5.00	100.00	100	75.00*	0.0	0.0
3.75	75.00	100	66.00*	0.0	0.0
2.50	50.00	100	63.00*	0.0	0.0
0.50	10.00	100	25.00	0.0	0.0
0.15	3.00	100	12.00	0.0	0.0
Sesame Oil		100	19.00	0.0	0.0

*Significantly different from solvent $p \leq 0.05$

MENTHOL
YOLK 0 HOURS

DOSE mg/egg	DOSE mg/kg	Number of Eggs	Percent Mortality*	Percent Abnormal Total	Percent Abnormal Structural
5.00	100.00	100	99.00*	0.0	0.0
3.75	75.00	100	88.00*	0.0	0.0
2.50	50.00	100	85.00*	0.0	0.0
0.50	10.00	100	71.00*	0.0	0.0
0.15	3.00	100	42.00	0.0	0.0
Sesame Oil		100	33.00	0.0	0.0
Control		100			

*Significantly different from solvent $p \leq 0.05$

MENTHOL
YOLK 96 HOURS

DOSE mg/egg	DOSE mg/kg	Number of Eggs	Percent Mortality*	Percent Abnormal Total	Percent Abnormal Structural
5.00	100.00	99	71.71 *	1.01	1.01
3.75	75.00	100	71.00 *	0.0	0.0
2.50	50.00	100	50.00 *	0.0	0.0
0.50	10.00	98	38.77	0.0	0.0
0.15	3.00	99	15.15 *	0.0	0.0
Sesame Oil		100	30.00	0.0	0.0

* Significantly different from solvent $p \leq 0.05$